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(54) NOVEL DRUGS FOR LIVER DISEASES

(57) Preventives and/or remedies for liver diseases, which comprise monocyte chemoattractant protein-1 (MCP-1) function inhibitors as active ingredients, respectively.

Administration of the MCP-1 function inhibitors brings about effects in preventing and/or remedying liver diseases.

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Description

Technical Field

[0001] This invention relates to novel drugs for liver diseases, and also to a novel preventive and/or remedial method for liver diseases.

Background Art

[0002] Chemokines are a group of proteins having migration activity for leukocytes and lymphocytes. From their structures, these chemokines can be divided roughly into four types. Those with the first and second cysteines arranged continuously are called "CC chemokines".

[0003] Monocyte chemoattractant protein-1 (MCP-1), one of the CC chemokines, was reported as a protein by itself, and at substantially the same time, its cDNA sequence was ascertained (J. Exp. Med., **169,** 1449-1459, 1989; J. Exp. Med., **169,** 1485-1490, 1989; FEBS lett., **244,** 487-493, 1989).

[0004] Receptors which recognize MCP-1 have already been identified, and their cDNAs have also been cloned (Proc. Natl. Acad. Sci. USA, **91**, 2752-2756, 1994; Biochem. Biophys. Res. Commun., **202**, 1156-1162, 1994). Nine types of receptors are now known as CC chemokine receptors, and the MCP-1 receptor is called "CCR2".

[0005] Rollins et al. reported that they prepared a variety of amino acid mutants of MCP-1 protein and some of the amino acid mutants were found to have lost cell migration activity (J. Biol. Chem., **269**, 15918-15924, 1994). Among these mutants, the mutant obtained by deleting the second to eighth amino acids as counted from the N terminal, that is, 7ND-MCP-1 has binding ability to CCR2, but does not provoke cell migration. As a dominant negative, on the other hand, 7ND-MCP-1 forms a dimer with wild-type MCP-1 and inhibits the function of MCP-1. Further, it is known that N-terminal deletions of chemokines are potent dominant negative inhibitors of chemokine-receptor interaction by forming heterodimers with the corresponding endogenous monomer of the chemokine and that these inhibitors are effective for the remedy of inflammations such as post-angioplasty restenosis, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, and chronic pulmonary inflammation, e.g., pulmonary fibrosis; autoimmune disease; and the like (JP-A-11506005).

[0006] Fibrosis of the liver is a morbidity in which destruction of the normal tissue structure, proliferation of fibroblasts and accumulation of extracellular matrices advance, and cirrhosis is a post-fibrosis disease. At present, no effective and safe remedial method has been established yet for these diseases. For example, various symptomatic treatments have been applied to cirrhosis, but cirrhosis advances to uncompensated cirrhosis, resulting in poor prognostic improvements.

[0007] An object of the present invention is to provide a novel preventive and/or remedy for a liver disease such as hepatic fibrosis or cirrhosis and further, a novel preventive and/or remedial method for such a liver disease.

Disclosure of the Invention

[0008] The present inventors have ascertained that 7ND-MCP-1 produced in myocytes by intramuscular injection of an expression vector containing 7ND-MCP-1 gene, into the femoral region of a model animal (rat) suppresses hepatic fibrosis and have found that MCP-1 function inhibitors are useful as preventives and/or remedies for liver diseases, leading to the completion of the present invention.

[0009] Described specifically, the present invention provides a preventive and/or remedy for a liver disease, comprising an MCP-1 function inhibitor as an active ingredient.

[0010] The present invention also provides a preventive and/or remedial method for a liver disease, which comprises administering a gene, which encodes an MCP-1 antagonist or an MCP-1 dominant negative, to an organism.

[0011] The present invention further provides a preventive and/or remedy composition for a liver disease, comprising an MCP-1 function inhibitor and a pharmaceutically acceptable carrier.

[0012] The present invention still further provides use of an MCP-1 function inhibitor for the manufacture of a preventive and/or remedy for a liver disease.

Best Modes for Carrying out the Invention

[0013] No particular limitation is imposed on the MCP-1 function inhibitor for use in the present invention insofar as it can inhibit the function of MCP-1 in the organism. Specific examples can include anti-MCP-1-antobodies (including polyclonals and monoclonals), MCP-1 antagonists (including proteins and non-protein, low molecular compounds), MCP-1 dominant negatives (including proteins and non-protein, low molecular compounds), and, when those capable of inhibiting the function of MCP-1 are proteins, also genes encoding such proteins. As these antibodies, antagonists,

dominant negatives, and encoding genes, a variety of antibodies, antagonists, dominant negatives and encoding genes are already known. Further, those available by methods known *per se* in the art are all usable in the present invention.

[0014] For example, anti-MCP-1 antibodies can be obtained by the procedure disclosed in J. Immunology, **147,** 2229-2233, 1991, while MCP-1 antagonists and MCP-1 dominant negatives are known from JP-A-11506005 and the like.

[0015] In the present invention, introduction of a gene encoding an MCP-1 function inhibitor is more preferred than administration of the MCP-1 function inhibitor as a protein to an organism, because the former allows the gene to remain longer in the organism (blood).

[0016] In the present invention, MCP-1 antagonists or MCP-1 dominant negatives are preferred, with 7ND-MCP-1 being particularly preferred. Further, genes encoding MCP-1 antagonists or MCP-1 dominant negatives are preferred, with a gene encoding 7ND-MCP-1 being particularly preferred. As the gene encoding 7ND-MCP-1, DNA having the base sequence indicated by SEQ ID NO: 1 of the Sequence Listing can be used. This DNA can be prepared by a genetic engineering procedure known *per se* in the art. Described specifically, from the base sequence of a DNA encoding the wild-type MCP-1 and indicated by SEQ. ID. NO: 2 of the Sequence Listing, the DNA can be prepared using PCR which employs a synthesis primer.

[0017] No particular limitation is imposed on an expression vector to be used for the expression of the gene in an organism insofar as it can exhibit its function. Illustrative are plasmid vectors such as pcDNA3, pEF-BOS and pXT1; and retrovirus vectors such as adenovirus vectors and Sendaivirus vectors. Upon constructing an expression vector, it is also possible to use a promoter or an enhancer. No particular limitation is imposed on the promoter or an enhance insofar as it functions in a host (organism). Examples of the promoter can include SV40 promoter, CMV promoter, HSV-TK, SRα, and RSV.

[0018] To have the gene expressed in the host (organism), liposomes are also usable. In this case, the gene may exist inside the liposomes, or inside or outside the lipid bilayer membranes which constitute the liposomes. A variety of liposome compositions are known to permit the expression of the gene in the host (organism).

[0019] To confirm production of 7ND-MCP-1 protein from the 7ND-MCP-1 gene introduced, it is only necessary to determine by ELISA whether or not the protein exists in serum.

[0020] Administration of the MCP-1 function inhibitor, which is an active ingredient of the preventive and/or remedy according to the present invention for a liver disease, to organisms of animals including human being can be conducted orally or parenterally. When the function inhibitor is a protein, parenteral administration is desired. As a parenteral administration method, injection can be mentioned. Injection can be performed directly to a diseased part (the liver) or to a part other than the liver, such as an artery, vein, muscle, skin or subcutaneous tissue. As a pharmaceutical preparation (preparation forms) for injecting the MCP-1 function inhibitor, an injection can be mentioned. This injection can be obtained by known pharmaceutical preparation manufacturing technology. Upon manufacturing the injection, one or more of known additives to pharmaceutical preparations can be added. Illustrative are isotonicities, buffers, preservatives, excipients, and soothing agents.

[0021] The dosage to each patient can be adequately determined depending on his or her condition, age, sex, weight and the like. For example, 0.1 to 1,000 mg (in the case of a protein) or 0.01 to 100 mg (in the case of a gene) may be administered once in 2 to 4 weeks.

Example

[0022] The present invention will next be described in further detail based on an example, although the present invention shall by no means be limited to the example.

(1) Construction and expression of 7ND-MCP-1

[0023] A plasmid vector encoding 7ND-MCP-1 was prepared by PCR using the pCDNA3 vector plasmid which encodes MCP-1 as a template. Each mutation was confirmed by a DNA sequence analysis from both directions. The resultant PCR product encoding 7ND-MCP-1 was inserted into the multicloning site of the pcDNA3 vector plasmid, the vector plasmid was transformed in *Escherichia coli*, and then, the plasmid DNA was purified using "Plasmid Giga Kit" (QIAGEN GmbH).

(2) Effect of 7ND-MCP-1 on dimethylnitrosamine-induced hepatic fibrosis in rats

[0024] Hepatic fibrotic model rats were prepared by intraperitoneally administering 1% dimethylnitrosamine (100 μL/100 g-rat weight) to male Wistar rats daily on three straight days a week for 4 weeks in total. Three days before administration of the mutated MCP-1 gene (7ND-MCP-1 gene), 0.25% bupivacaine hydrochloride (100 μL/100 g-rat weight) was intramuscularly injected to the right femoral muscles of the rats to conduct pretreatment for increased

efficiency of gene introduction.

[0025] The mutated MCP-1 gene (7ND-MCP-1 gene) was intramuscularly injected (100 μg DNA [1 μg/l μL]/100 g-rat weight) into the pretreated parts on the day of start of the dimethylnitrosamine administration. To a control group, the vector DNA was administered in the same amount. The mutated MCP-1 gene (7ND-MCP-1 gene) was readministered in the same amount as mentioned above to the left femoral muscles of the rats on the 12^{th} day of the dimethylnitrosamine administration. Further, three days before the readministration (on the 12^{th} day of the dimethylnitrosamine administration), the above-mentioned pretreatment with bupivacaine hydrochloride was applied likewise. To the control group, the vector DNA was also administered similarly.

[0026] Twenty-eight (28) days later, the liver was enucleated, and its weight, and levels of tissue fibrosis and tissue hydroxyproline were measured. The fibrosis level was determined by staining a fibrosed part in accordance with the Masson's trichrome staining. The level of tissue hydroxyproline was measured by HPLC.

[0027] As a result, the liver weight was 10.42 ± 4.01 g ($p<0.05$ according to the Mann-Whitney significant test) and ± in the group administered with the mutated MCP-1 gene (7ND-MCP-1 gene) as opposed to 4.95 ± 2.00 g in the control group, and the fibrosis level was ±, as opposed to +++ in the control group. A pronounced hepatic fibrosis inhibiting effect was observed.

[0028] The tissue hydroxyproline level was 28.2 ± 9.12 μmol/g-rat liver weight ($p<0.05$ according to the Mann-Whitney significant test) in the group administered with the mutated MCP-1 gene (7ND-MCP-1 gene) as opposed to 186.75 ± 130.78 μmol/g-rat liver weight) in the control group. A significant lowering effect on the hydroxyproline in the liver tissue by the administration of the mutated MCP-1 gene (7ND-MCP-1 gene) was hence observed.

Industrial Applicability

[0029] As evident from the Example, the MCP-1 function inhibitors are useful as preventives and/or remedies for liver diseases such as hepatic fibrosis and cirrhosis.

SEQUENCE LISTING

<110> EGASHIRA KENSUKE;DAIICHI PHARMACEUTICAL CO., LTD

<120> Novel hepatic disease agent

<130> DS0057

<150> JP 2000-310604

<151> 2000-10-11

<160> 2

<210> 1

<211> 279

<212> DNA

<213> Homo sapiens

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ctcgcgagct atagaagaat caccagcagc aagtgtccca aagaagctgt gatcttcaag 120

accattgtgg ccaaggagat ctgtgctgac cccaagcaga agtgggttca ggattccatg 180

gaccacctgg acaagcaaac ccaaactccg aagacttga 279

<210> 2

<211> 300

<212> DNA

<213> Homo sapiens

<300>

<301> Yoshimura, T., Yuhki, N., Moore, S.K., Appella, E., Lerman, M.I., Leonard, E.J.

<302> Human monocyte chemoattractant protein-1 (MCP-1): full length DNA cloning, expression in mitten-stimulated blood mononuclear leukocytes, and sequence similarity to mouse competence gene JE.

<303> FEBS Letters

<304> 244

<305> 2

<306> 487-493

<307> 1989-02

<400> 2

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aggaagatct cagtgcagag gctcgcgagc tatagaagaa tcaccagcag caagtgtccc 180

aaagaagctg tgatcttcaa gaccattgtg gccaaggaga tctgtgctga ccccaagcag 240

aagtgggttc aggattccat ggaccacctg gacaagcaaa cccaaactcc gaagacttga 300

Claims

1. A preventive and/or remedy for a liver disease, comprising a monocyte chemoattractant protein-1 (MCP-1) function inhibitor as an active ingredient.

2. A preventive and/or remedy, wherein said MCP-1 function inhibitor comprises one or more inhibitors selected from anti-MCP-1 antibodies, MCP-1 antagonists, MCP-1 dominant negatives and encoding genes thereof.

3. A preventive and/or remedy according to claim 2, wherein said genes encoding said MCP-1 dominant negatives are indicated by SEQ ID NO: 1 of the Sequence Listing.

4. A preventive and/or remedial method for a liver disease, which comprises administering a gene, which encodes

an MCP-1 dominant negative, to an organism.

5. A preventive and/or remedial method according to claim 4, wherein said gene is represented by SEQ ID NO: 1 of Sequence Listing.

6. A preventive and/or remedial method according to claim 5, wherein said gene is administered to said organism at a site other than the liver.

7. A preventive and/or remedial method according to claim 6, wherein said site other than the liver is a muscle.

8. A preventive and/or remedy composition for a liver disease, comprising an MCP-1 function inhibitor and a pharmaceutically acceptable carrier.

9. Use of an MCP-1 function inhibitor for the manufacture of a preventive and/or remedy for a liver disease.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP01/08552

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] A61K45/00, A61K48/00, A61P43/00, 1/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] A61K45/00, A61K48/00, A61P43/00, 1/16

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Jitsuyo Shinan Koho 1940-1992 Toroku Jitsuyo Shinan Koho 1994-1996
Kokai Jitsuyo Shinan Koho 1971-1992 Jitsuyo Shinan Toroku Koho 1996-2001

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAPLUS(STN), BIOSIS(STN), MEDLINE(STN), EMBASE(STN),
JICST(JOIS), Genbank/EMBL/DDBJ/GeneSeq

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Czaja M. J. et. al., Monocyte chemoattractant protein 1 (MCP-1) expression occurs in toxic rat liver injury and human liver disease., JOURNAL OF LEUKOCYTE BIOLOGY, 1994, Vol.55, No.1, pp.120-126, whole document, especially, p.124	1,8,9 2,3
X Y	Marra F. et. al., Monocyte chemotactic protein-1 as a chemoattractant for human hepatic stellate cells., HEPATOLOGY, Vol.29, No.1, pp.140-148, whole document, especially DISCUSSION	1,8,9 2,3
X Y	Marra F. et. al., Increased expression of monocyte chemotactic protein-1 during active hepatic fibrogenesis: correlation with monocyte infiltration., AMERICAN JOURNAL OF PATHOLOGY, 1998, Vol.152, No.2, pp.423-430, whole document	1,8,9 2,3
Y	WO 96/38559 A1 (DANA FARBER CANCER INST. INC.), 05 December, 1996 (05.12.96), whole document & US 5705360 A & EP 828833 A1 & JP 11-506005 A	1-3,8,9

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 December, 2001 (12.12.01)	25 December, 2001 (25.12.01)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP01/08552

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 2000-239182 A (Mitsubishi Chemical Corporation), 05 September, 2000 (05.09.00), Full text; especially, Claims, Par. No. [0004] (Family: none)	1,8,9
Y	JP 11-60502 A (Teijin Limited), 02 March, 1999 (02.03.99), Full text (Family: none)	1,2,8,9
Y	JP 8-119934 A (Teijin Limited), 14 May, 1996 (14.05.96), Full text (Family: none)	1,2,8,9
Y	JP 9-67399 A (Mitsui Toatsu Chemicals Inc.), 11 March, 1997 (11.03.97), Full text (Family: none)	1,2,8,9
Y	WO 98/6703 A1 (WARNER LAMBERT CO.), 19 February, 1998 (19.02.98), whole document & EP 927167 A1 & JP 2000-516611 A & US 6184235 A	1,2,8,9
Y	WO 99/7678 A1 (ZENECA LTD.), 18 February, 1999 (18.02.99), whole document & EP 1001935 A1 & US 6288103 A & JP 2001-512716 A	1,2,8,9
Y	WO 95/13295 A1 (DANA FARBER CANCER INST. INC.), 18 May, 1995 (18.05.95), whole document & EP 725794 A1 & JP 9-505053 A	1,2,8,9
Y	WO 96/23068 A1 (GLAXO GROUP LTD.), 01 August, 1996 (01.08.96), whole document & EP 805859 A1 & JP 10-513046 A & US 6150132 A	1,2,8,9

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP01/08552

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 4-7
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 4 to 7 pertain to methods for treatment of the human body by therapy and thus relate to a subject matter which this International Searching Authority is not required, under the provisions of Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP01/08552

Claims 1, 2, 8 and 9 relate to preventives and/or remedies for liver diseases containing as the active ingredient compounds defined by desired properties, i.e., "monocyte chemoattractant protein-1 (MCP-1) function inhibitors", "MCP-1 antagonists" and "MCP-1 dominant negatives and genes encoding the same". Although it seems that any compounds having these properties fall within the scopes of claims 1, 2, 8 and 9, it is recognized that only small part of the claimed compounds are exclusively supported by the description under the provision of Article 6 of the PCT and disclosed therein under the provision of Article 5 of the PCT.

Even though the common technical knowledge at the point of the application is taken into consideration, it cannot be recognized that the scope of compounds corresponding to "monocyte chemoattractant protein-1 (MCP-1) function inhibitors", "MCP-1 antagonists" and "MCP-1 dominant negatives and genes encoding the same" could be specified. Thus, the above claims also fail to satisfy the requirement of clearness as defined in Article 6 of the PCT.

Therefore, the search has been practiced mainly on the relationship between the MCP-1 function inhibition and liver diseases and preventives and/or remedies for liver diseases containing specific components cited in the description as the active ingredient.

On the other hand, claim 3 has been completely searched.

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